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14. ABSTRACT

We have performed the spectral analysis of the sleep EEG in what is likely the largest group of subjects ever assembled and have demonstrated marked effects of age, sex and ethnicity. In these healthy non-obese volunteers who had demographics similar to the demographics of active duty Army personnel, BMI did not impact SWA. Between the ages of 21 and 30, there are large inter-individual variations in SWA that are only partly predicted by sex and ethnicity. Our hypothesis is that these individual differences may partly predict diabetes risk. We have demonstrated the feasibility of the clinical study and successfully obtained all planned assessments. The effort to increase our data base will be valuable to the entire project. While progressing through the analytical and experimental work, we have prepared two reviews on sleep loss and the risk of obesity and diabetes.

15. SUBJECT TERMS

Sleep, Diabetes, Slow-Wave activity, Weight Gain

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Introduction

Chronic partial sleep loss, due to bedtime restriction, is a hallmark of modern society and highly prevalent in active duty army personnel. During the past few years, evidence from laboratory and epidemiological studies has indicated that decreased sleep duration has an adverse effect on glucose regulation and on the neuro-endocrine control of appetite (1-3). Taken together, the findings suggest that chronic partial sleep deprivation may be involved in the current epidemic of obesity and diabetes. Our group has strong evidence for the existence of large individual differences in metabolic as well as cognitive vulnerability to sleep loss. We have recently obtained preliminary data in a small group of young men that suggest that a specific heritable trait of the sleep electroencephalogram (EEG), known as slow-wave activity (SWA), accounts for the majority of individual variability in the adverse effects of sleep loss on diabetes risk.

The objectives are to identify SWA as a predictor of diabetes risk in a subject population with a gender, ethnic and age distribution similar to that of active duty army personnel and to test the hypothesis that individuals with low SWA are at much higher risk to develop diabetes following chronic partial sleep restriction than those with higher SWA. The studies will also explore the potential relationships between individual differences in diabetes risk following sleep loss and individual differences in risk of weight gain and in the magnitude of cognitive deficits.

Body of Report

Overview

The Statement of Work for the first 3 years of the award included:

Task 1: Testing role of EEG SWA as predictor of individual differences in baseline glucose disposition index (Months 1-6):

- Perform spectral analysis of sleep EEG for 56 subjects in whom EEG recordings and glucose and insulin levels during intravenous glucose tolerance testing are presently available
- Re-run minimal model analysis of ivGTT results for 56 subjects already tested
- Recruit and study 7 additional subjects using currently IRB-approved protocol in order to match gender, age and ethnic distribution of active army personnel
- Test the hypothesis that levels of SWA in the sleep EEG are a significant predictor of beta-cell responsiveness and insulin sensitivity after controlling for gender, age, BMI and ethnicity-based diabetes risk.
- Define lower and upper thirtiles of slow-wave activity associated, respectively, with putatively high and low diabetes risk during sleep curtailment

Task 2: preparation of clinical study of diabetes risk during sleep curtailment (Months 1-6):

- Submit protocol to and obtain approval from University of Chicago Institutional Review Board and Clinical Research Center
- Import Walter Reed Army Institute of Research battery of neurobehavioral testing and train personnel in its use
- Design database.

Task 3: Completion of clinical study of diabetes risk during sleep curtailment in 32 individuals (Mos. 7-42 – one study per month):

- Recruit and screen 60-70 individuals to enroll 16 individuals with SWA in lower third and 16 individuals with SWA in upper third of distribution with both groups matched for gender distribution
- Complete study in two groups of 16 individuals
- Generate individual data analysis and enter in database.

Research accomplishments associated with Task 1

Experimental work

Was completed in year 2 and included in the 2009 Annual Report.

Analytical work

In our annual report for year 1, we indicated that we had performed the spectral analysis of the sleep EEG for 44 of the 56 subjects for whom the recordings were readily available at the time of the application. We had also indicated that if the cut offs points for low and high SWA, respectively, were derived from a larger data base of recordings obtained in subjects with a sex, age, ethnic and BMI distribution similar to that of active duty Army personnel, these cut off points would have greater precision. Since the inception of this project, we have continually updated our data base of recordings accumulated in our laboratory. These data have been obtained as a result of baseline testing of volunteers entering IRB-approved NIH-funded studies where sleep duration or quality was subsequently manipulated. In each individual, baseline polysomnography was performed after at least 2 nights of normal bedtimes (8-9 hours) and a frequently sampled ivGTT was performed on the following day. In our 2009 report, we reported the EEG spectral analysis for 128 recordings, a data set more than twice as large than that proposed in our original application (128 versus 63). To date, we have 191 volunteers in the data base, with EEG spectral analysis in 147 subjects. The gender, age and ethnic distribution of the subjects are consistent with recommendations for active duty Army personnel. The lower and upper thirtiles remain similar to what we reported in 2009: the lower thirtile is now 718 μV^2 and the upper thirtile is now 1307 μV^2. Corresponding values in 2009 were 728 μV^2 and 1311 μV^2, respectively. In the final year of this award, we will therefore focus our efforts on the experimental work in Task 3 rather than continue to refine our estimations of cut off points for low and high SWA.

Minimal model analysis of the results of intravenous glucose tolerance testing (ivGTT) for 112 of the 147 subjects has been performed using standardized parameters. We will run the last 35 subjects in the first quarter of Year 4 and run general linear model analyses to determine the contribution of individual differences in SWA in the risk of type 2 diabetes, as assessed by the disposition index derived from the minimal model analysis of the ivGTT.

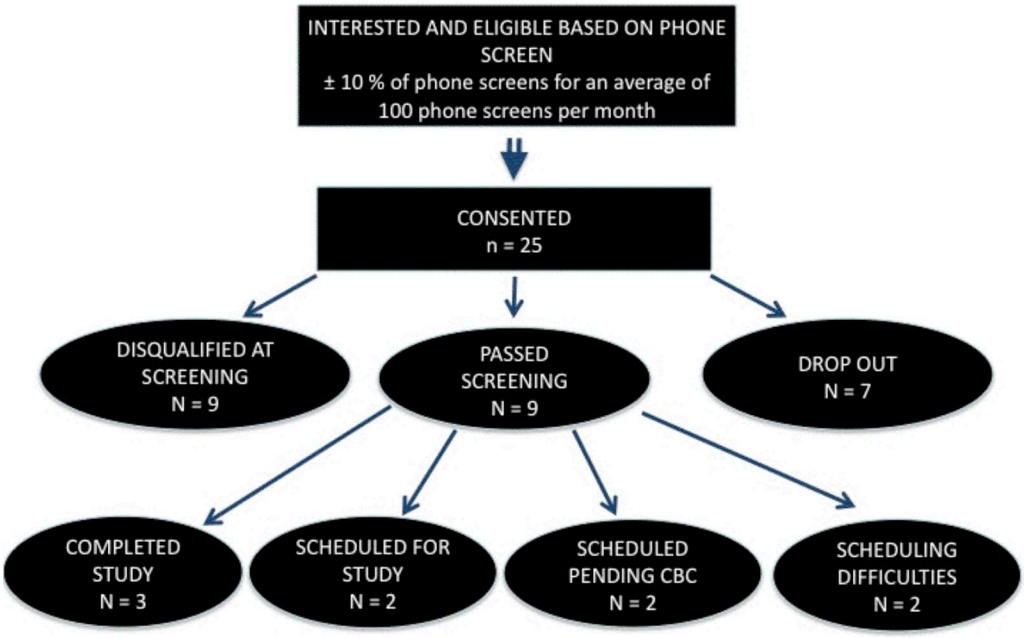
A communication on the work accomplished under Task 2 was presented by the Principal Investigator at the Military Health Forum 2009.

***Research accomplishments associated with* Task 2**

This work has been reported in the Annual report for year 2. The Task is completed.

***Research accomplishments associated with* Task 3**

A flow chart showing the progress of the study is shown below (Figure 1).

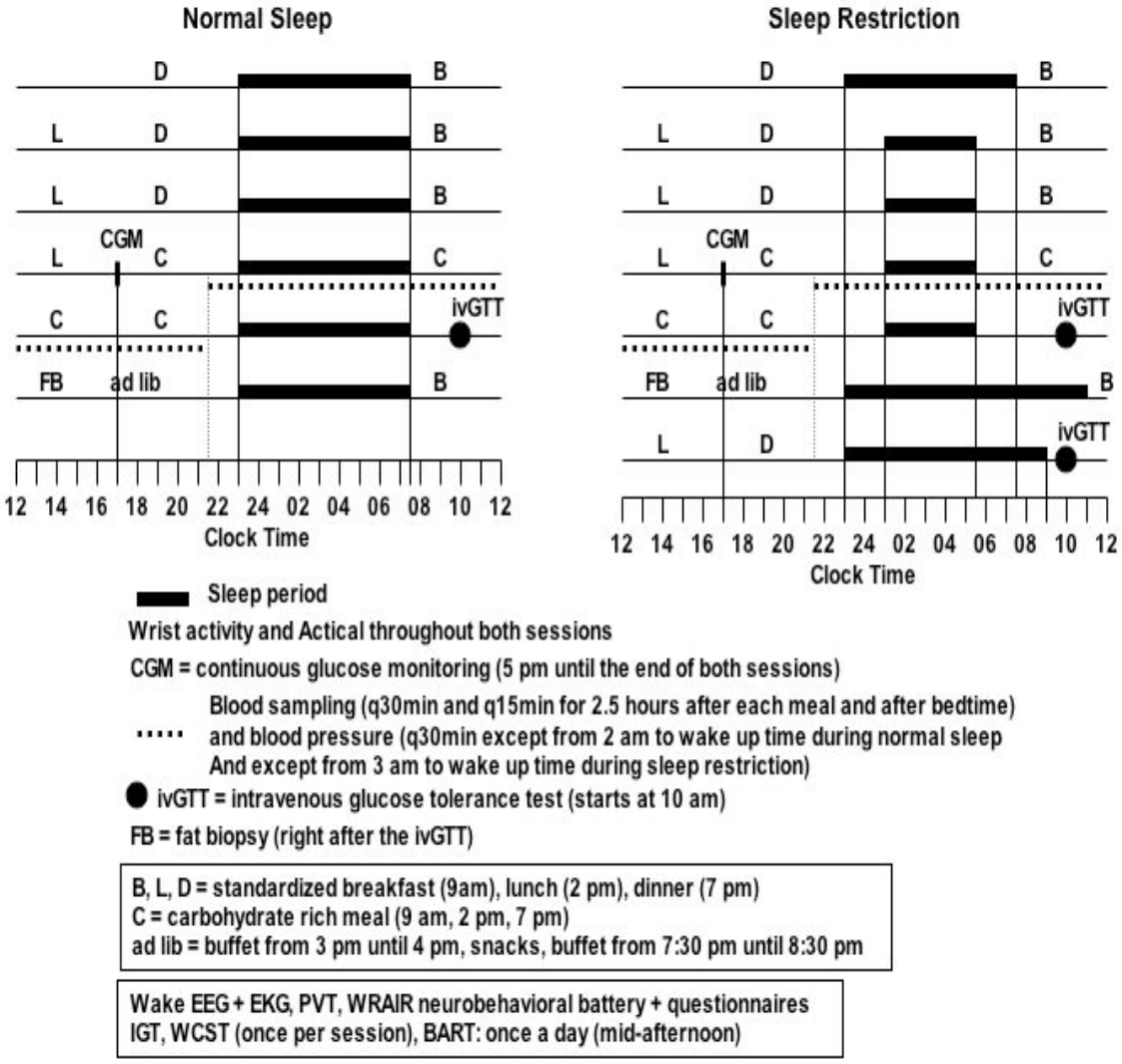


- Figure 1 -

Scheduling these week long studies has been a real issue in our Clinical Research Center due to NIH budget cuts and ~~but~~ we have unfortunately not kept track of subjects who were interested to participate but could not accommodate our open dates. We have previously reported these problems.

Three subjects have completed the entire protocol and two additional subjects have passed the screening tests and are scheduled for the study in September and October

2010, respectively. Two additional subjects have passed all screening tests but had low CBC and are taking iron tablets in an effort to meet inclusion criteria based on hematocrit levels. Recruitment efforts including posting flyers, putting adds in local free newspapers and using word of mouth are continuing. We are vigorously recruiting and approximately 100 individuals call our recruitment line on a monthly basis. We return all phone calls at least twice, leaving messages. Unfortunately, the majority (90%) of subjects who have expressed an initial interest do not follow up. The phone log of our subjects recruiter for the month of June 2010 is shown below as an example.



- Figure 2 -

We have not experienced difficulties running the studies.

We plan to run 10 to 12 studies in year 4 to have data on 13-15 subjects at the end of year 4. This increased pace is possible because our Sleep Research Laboratory is now approved for invasive procedures and part of the studies are run in our laboratory rather than in the Clinical Resource Center (CRC). While this development (which was facilitated by the fact that the PI was recruited by Harvard Medical School and the University of Chicago offered resources to bring the laboratory in compliance with hospital standards as part of the retention package) is accelerating the pace of studies, budgetary issues become problematic because the CRC was covering part of the inpatient costs while we have to cover all costs incurred in the laboratory.

However, we found a way to cut some of the costs allocated for assays. In the past year, we have piloted a new procedure for hormonal measurements which we propose

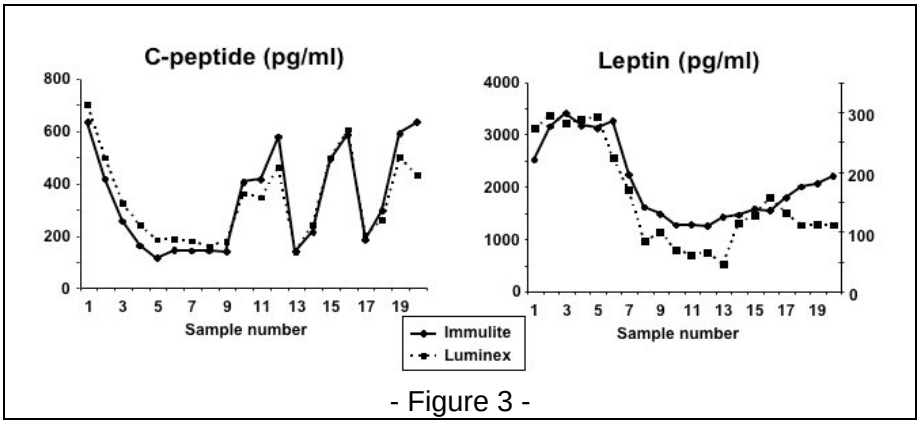
to use in the remainder of this project. As a reminder, in the original proposal, we included assay of glucose, insulin and C-peptide on all samples collected during the 24-h study (one per study session) and during the ivGTT and OGTT. This represents a total of 252 samples per subject completed. These assays were projected to be at no cost to the project because glucose levels were measured at the bed site by the CRC staff at no charge and the costs of the insulin and C-peptide assays were performed at no charge by the University of Chicago Diabetes Research and Training Center, a facility funded by NIH/NIDDK. Since the initiation of the project, both the CRC and DRTC have been under budgetary pressure from NIH and have started charging a nominal fee for assays. These fees are $1.20 for glucose, $2.56 for insulin and $2.56 for C-peptide. We had stated in the approved protocol that we would assay glucose, insulin and C-peptide on all samples and seek additional funding to assay other hormones that play a major role in appetite regulation and glucose homeostasis and have been shown by multiple groups, including us, to be affected by sleep deprivation. So far, we have indeed measured levels of leptin and ghrelin on all samples. These two assays have a cost of $9.91 each using our existing methods. Thus, to complete 12 more subjects in the remainder of the grant period, we would need to dedicate 252 x ($2.56 x 2) = $1,290 per subject for insulin and C-peptide and 138 x ($9.91 x 2) = $2,735 per subject for leptin and ghrelin. The new procedure described below will cut these cost by 75%.

This technology will also increase the scientific output of the project because more hormones relevant to the interaction between sleep and metabolism will be measured. The Luminex procedure is a well-validated adaptation of the immunometric principle wherein a capture antibody is attached to small, 5 – 10 micron, polystyrene beads. A second, biotinylated antibody completes the “sandwich” and an indicator (phycoerythrin) associated with streptavidin is associated to the complex via the biotin. The beads are washed to remove unbound signal, resuspended and pushed through a narrow tube where each individual bead of unique antibody characteristic is identified by the ratio of dyes. Co-incidentally, the indicator signal is detected and quantified. Readings for each bead type (e.g. 75 insulin-specific beads) are accumulated and the average compared to a standard curve obtained with known amounts of analyte.

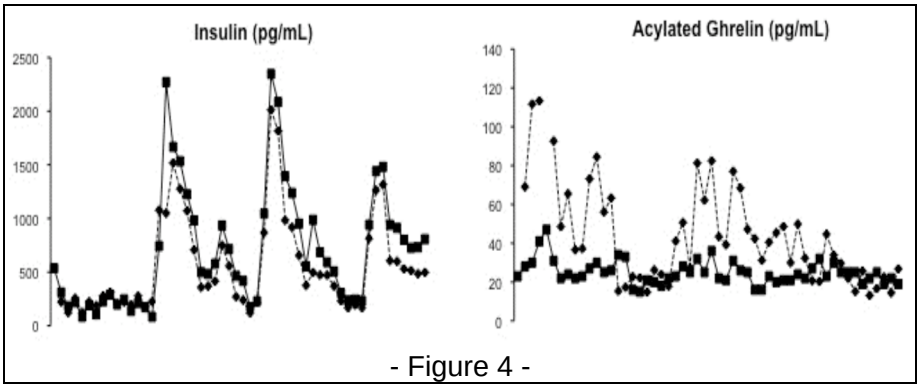
The benefits of the Luminex technology are:

1) Multiple substances measured simultaneously avoiding concerns of unequal pipetting or inconsistent sample freeze/thaw history. We have requested from the manufacturer a custom supplied panel of assays that includes insulin, pancreatic polypeptide (PP), leptin, active ghrelin, as well as the two incretin hormones GLP-1 and GIP. We already had tested a more limited panel with just C-peptide and leptin. The cost of assaying 6 hormones per sample is roughly $3, including disposable supplies and the fee for the use of the reader in the Core facility.
2) Improved sensitivity of the measurement as compared to our current Immulite procedure.
3) Small volumes required, typically 25 µl/well: this is a major advantage in terms of decrease in blood volume needed from each volunteer. Currently, to assay insulin, C-peptide, ghrelin and leptin, a minimum of 0.4 ml is needed.

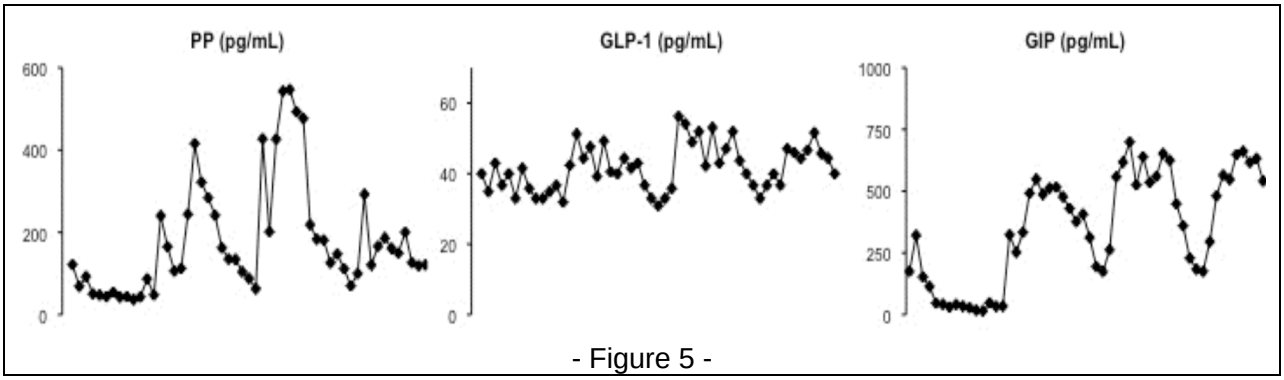
Figure 3 below shows a comparison of the two methods for 24-h profiles of C-peptide and leptin. The results are essentially superimposable. Figure 4 shows a similar comparison for insulin and ghrelin. The Luminex assay actually quantifies the meal-related excursions of ghrelin better than our current assay (in our protocol, meals are given at 9:00, 14:00 and 19:00). Mean levels of ghrelin differ between the two assays (as they do when one compares two different RIAs) but this is not relevant for our study where all comparisons are within subject. Lastly, Figure 5 shows the 24-h profiles of PP, GLP-1 and GIP. All three hormones show the expected post-prandial profiles. Running these important additional assays does not increase the cost, which remains at $3 per plate.



- Figure 3 -



- Figure 4 -



- Figure 5 -

The total cost of using the Luminex assay is, for 12 subjects completed, $9,072 as compared to $48,300 with our current methods.

We will request a no cost extension at the end of year 4 to continue to run studies which will be facilitated by the savings on assays. We also propose to merge our data with de-identified data from subjects studied between 2006 and 2010 in our laboratory using a similar protocol (but involving more output measures and a 3rd experimental condition, i.e. slow wave sleep suppression by acoustic stimuli). Twenty subjects have completed the 3 conditions of that protocol (baseline, sleep restriction and slow wave sleep suppression). We would extract from that database the subjects that are either high or low slow-wave activity (approximately 13-14 subjects) for the baseline and sleep restricted conditions and for the variables that are part of the outcome measures of the present project. After consultation with our IRB, this will be allowed after we apply for and obtain a waiver of consent for these de-identified data. This merge of database would bring the total amount of subjects included in our analysis to approximately 30 by the middle of the year of no cost extension, i.e. very close to the target proposed in the original application (n=32). We would then dedicate the remainder of the no cost extension to data analysis. This appears to be the best strategy to achieve our original goals within the budgetary constraints of our CRC (which developed after our project was initiated) and of the PRMRP (where a budget supplement is not feasible).

Key Research Accomplishments

We have demonstrated the feasibility of the clinical study and successfully obtained all planned assessments. We have increased our recruitment efforts.

The effort to increase our database will be valuable to the entire project.

We have piloted a new analytical procedure for hormonal measurements which will result in large budgetary savings and increase the scientific output of the project.

While progressing through the analytical and experimental work, we have prepared two reviews on sleep loss and the risk of obesity and diabetes (see appendices).

Reportable Outcomes

We presented our preliminary data at the Military Health Forum 2009. Two review articles have been published in 2010.

Conclusions

The work has made substantial progress during the past year. We propose a realistic plan to achieve the goals of the original application despite the budgetary hurdles that developed since 2007. The preparation of review articles has indicated that the body of evidence supporting the hypotheses to be tested in the present project has clearly increased.

Publications.

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Leproult R and **Van Cauter E**. Role of sleep and sleep loss in hormonal release and metabolism.

Endocr. Dev. 2010; 17: 11-21. Epub 2009 Nov 24. PMID: 19955752.